ANTI-FERTILITY ACTIVITY OF MONTELUKAST IN FEMALE ALBINO WISTAR RATS

A dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY

CHENNAI- 600 032.

In partial fulfillment of the requirements for the award of Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted

By

HERALD SAMUEL YESUBALAN K

(Reg No: 261325953)

Under the guidance of

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DEPARTMENT OF PHARMACOLOGY

EDAYATHANGUDY.G.S PILLAY COLLEGE OF PHARMACY

NAGAPATTINAM-611002

OCTOBER 2015

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CERTIFICATE

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CHAPTER-1

INTRODUCTION

Anti-fertility agents are the agents, which prevents fertility by interfering with various normal reproductive mechanisms, in both males and females. Development of newer methods/agents for fertility control and research in this direction are imperative particularly the developing nations. If an ideal contraceptive were available, that contraceptive would be 100% effective, safe and easy to use; its effect would be reversible.[1]

The world population explosion has pointed out the need for new, effective, and safe contraceptive agents or methods for maximum protection. Side effects of synthetics on normal and natural human body are much more aggressive and unpredictable at prolonged use as long as one which gender expects to use them. Now time is alarming us to think us some alternative in the field of contraception.[2]

India within, few years of time span will be the leading country as far as the population growth is concerned. Since the population rising tremendously, this may affect drastically the economic growth of India. Family planning has been promoted through several methods of contraception, but due to side effect produced by the use steroidal contraceptive and use of abortifaciant drugs. There is a need of drug which is effective with lesser side effects.[3]

Approximately 48.2% of couples of 15 to 49 years of age practice family planning methods in India. Female sterilization accounts for 34.2%, with male sterilization declining from 3.4% in 1992–93 to 1.9% in 1998–99. Use of the condom increased to 3.1% from 2.4%. There is an urgent need for research to develop new contraceptive modalities especially for men and also for women and to make existing methods more safe, affordable and acceptable.[4]

Arachidonic acid, a fatty acid synthesized from dietary linoleic acid, metabolizes in the body by cyclooxygenase (COX) and lipoxygenase (LOX) pathways. Metabolites of arachidonic acid are called eicosanoids. The COX pathway leads to prostaglandin and thrombaxane production and the LOX pathway leads to the leukotrienes and hydroxyeicosatetraenoic acids.[5] The leukotrienes (LTs) are 5-lipoxygenase metabolites of arachidonic acid. The synthesis and release of (LTs) have been demonstrated in many cells and organs and LTs are considered to be normal product of continuous metabolism of arachidonic acid.[6]

Montelukast, a selective reversible cysteinyl leukotriene D_4-receptor (LTD_4 receptor) antagonist is currently available in the market for the treatment of asthma. It was found to have the gastro protective and antioxidant effects on indomethacin-induced gastric ulcer in rats,[7] prophylactic potential against mild colitis induced by dextran sulphate sodium in rats[8] and protective effect on smoking induced lung injury in *Wistar* rats.[9]

The lipoxygenase products (leukotrienes) have been demonstrated in many mammalian tissues including humans. They are widely distributed in the lungs, gut, uterus, kidneys, skin, heart and the liver. Their roles as mediators of inflammation have made them therapeutic targets. The potential use of lipoxygenase products in the management of primary dysmenorrhoea (especially in patients who are not responding to the traditional treatment using PG synthetase inhibitors) and possibly also in cases of endometriosis.[10]

The concentrations of PGE, PGF, LTB and LTC4/D/E increase in the ovary of PMSG-primed immature rats after the ovulatory process has been initiated by hCG administration. Since differences in the patterns of temporal changes of the various eicosanoids during ovulation are seen, we suggest that each eicosanoid plays a distinct role in the process of follicular rupture.[11]

Pharmacological inhibition of an alternative lipoxygenase (LOX) pathway has been shown to cause defective ovulation. The expression of ALOX5 and ALOX12 in the preovulatory follicle and that inhibiting the early phase LOX activity led to reduction in preovulatory PTGS2 induction and PGE2 production and thus defective ovulation. These findings may help to understand the mechanism for the involvement of the LOX pathway in LH-triggered ovulatory response, at least the induction of PTGS2 activity, in rats.[12] LTB4 may play a role in the ovulatory pathways of the rat ovary and that LTB4 may carry out its effects via MMP-2.[13]

NDGA appears to be an inhibitor of ovulation in the rat. LTB 4 reversed the ovulation inhibition by NDGA, suggesting a role of this LT in intraovarian ovulatory changes. [14]

LTs in uterine functions during implantation processes and decidualization has been reported.[15, 16] The presence of the lipoxygenase pathways in the pre-implantation rabbit uterus and blastocyst, their differential operation in various compartments of the uterus on various days of early pregnancy suggests an integrated role for these mediators in embryo-uterine interaction during implantation.[17] Mouse and human spermatozoa require cysteinyl leukotriene activity for both fertilization and oocyte penetration.[18]

Lipoxygenase metabolites may be involved in human parturition. Role for 5-LOX and FLAP in the control of parturition at term, and also suggest an involvement earlier in pregnancy.[19] HSD11B2 in human placental trophoblasts is decreased by progesterone and increased by inhibition of endogenous LOX metabolites, and that a component of the effect of LOX metabolites on HSD11B2 is mediated by their stimulation of endogenous progesterone output.[20] LTs are required for the induction and progression of decidualization.[21]

All above literatures proved that leukotrienes are involved in normal reproduction. If these leukotrienes are blocked or their receptor are blocked it may produce negative effect in reproduction. Based on this hypothesis in this study we have evaluated anti-implantation activity of montelukast a cysteinyl leukotriene receptor antagonist in female albino *Wistar* rats.

CHAPTER-2
LITERATURE REVIEW

Anti-fertility agents are the agents, which prevents fertility by interfering with various normal reproductive mechanisms, in both males and females. Development of newer methods/agents for fertility control and research in this direction are imperative particularly the developing nations. If an ideal contraceptive were available, that contraceptive would be 100% effective, safe and easy to use; its effect would be reversible. It should be aesthetically and personally acceptable in a variety of social, political and religious setting. It would be suitable culturally in terms of local attitudes concerning sexuality, reproduction; menstruation and the roles and responsibilities of men and women, and it would be applicable in terms of the health status of widely differing populations. It would be affordable, readily available and legal. Finally it would be appropriate for use at all stages of reproduction.[1]

2.1 Available methods of contraception:

In the earlier part of present century, methods of contraception used (condoms, diaphragm, spermicidal creams, foam tablets etc.) were intimately related to sexual intercourse, therefore, despised by most couples and they also have higher failure rate. Hence hormonal contraceptives are widely used, with 100% confidence and complete return to fertility on discontinuation.[22]

The most widely used methods of contraception are.[22, 23]

2.1.1 Hormonal contraceptives

a. Oral contraceptives

1. Combined pill

2. Phase regimens

3. Minipill (luteal supplement)

4. Post coital (emergency contraception)

b. Injectable contraceptives

c. Implants

2.1.2 Abortifacients

a. Anti-progesterone (mifepristone or RU486)

b. Prostaglandins

The control of fertility is frequent concern of women and health care providers the world over. Unfortunately there are still no 100% safe and effective contraceptive methods besides complete abstinence. One reason why some women may be reluctant to take oral contraceptives consistently and correctly is a fear of possible adverse effects listed below. [23, 24, 25]

Cardiovascular disease: Myocardial infraction, stroke, hypertension, venous thromboembolism, cerebrovascular insults, raise in HDL/LD ratio and high blood pressure.

Cancer: Uterine cancer, ovarian cancer, endometrial cancer, breast cancer, cervical cancer, hepatoma, focal nodular hyperplasias, liver cell adenoma, hepatocellular cancer and genital carcinoma.

Metabolic and endocrine effects: Diabetes or impaired glucose tolerance, gall stone and cholecystitis.

Down-regulating bone metabolism.

Others: Nausea, vomiting, headache, migraine, breakthrough bleeding or spotting, breast discomfort, weight gain, acne, increase body hair, chloasma, mood swings and abdominal distension.

2.2 The physiology of female reproductive system:

The principal organs of the human female reproductive tract are the ovaries, the fallopian tubes, the uterus, and the vagina. Reproduction begins with the development of ova in the ovaries, a single ovum is expelled from an ovarian follicle into the abdominal cavity in the middle of each monthly sexual cycle, this ovum then passes through one of the fallopian tubes into the uterus, and if it has been fertilized by a sperm, it implants in the uterus where it develops into a fetus, a placenta and fetal membranes.

The ovarian hormones plays an important role in pregnancy, the different ovarian hormones are estrogen, and progesterone. Estrogen mainly secreted by the growing follicles and progesterone mainly secreted by the corpus lutea.

When pregnancy occurs the ordinary ovarian cycle is suspended and the hormone relaxin secreted by corpus lutea and placenta ensures uterine quiescence and prevents early abortion of the pregnancy.

2.3 Female Hormonal system:

- A hypothalamic-releasing hormone, luteinizing hormone releasing hormone (LHRH).
- The anterior pituitary hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), both of which are secreted in response to the releasing hormone from the hypothalamus.
- The ovarian hormones, estrogen and progesterone, which are secreted by the ovaries in response to the two hormones from the anterior pituitary gland.

The various hormones are not secreted in constant amounts throughout the female monthly sexual cycle, but instead are secreted at drastically different rates during different parts of the cycle.[26]

2.4 Estrogens:

Estrogens are a class of steroid hormones linked principally with the control of female sex organ responsiveness and of reproduction. The important endogenous estrogens are 7 ß-estradiol, estrone and estriol. The most potent biogenic form is 17 ß estradiol. Estrogens are biosynthesized in the ovary.

Estrogenic compounds derived from plants are termed phytoestrogens. Themajor chemical groups of phyto-estrogens are classified as flavonoids, (flavones, flavanones and isoflavanoids) coumastrans, lignans and myoestrogens.

These compounds interacting with the estrogenic receptor exert estrogenic activity, such as uterotropic effect, sterility or disruption of normal reproductive processes occurs in farm animals grazing in postures with plant sources high in phyto estrogens. In humans cancer protective properties have been associated with phyto- estrogens.[27]

2.4.1 Physiology of estrogens:

Estrogens are required for the normal maturation of the female. They stimulate the development of vagina, uterus and uterine tube as well as the secondary sexual characteristics. They stimulate development of stroma and ductal growth in the breast. They contribute to the growth of the axillary and pubic hair and later the distribution of body fat. Estrogens also play an important role in the development of endometrial lining. An estrogen stimulates the synthesis of enzymes and growth factors leading to uterine growth and differentiation. In the liver estrogens alter the circulating levels of proteins such as transcorin (CBG) thyroxine-binding globin (TBG) sex hormone-binding globin (SHBG), tranferrin, renin substrate and fibrinogen. This leads to increased circulating levels of thyroxine, estrogen testosterone, iron, copper and other substances. Estrogens enhance the coagulability of blood. Alteration in the composition of the plasma lipids caused by estrogens is characterized by an

increase in the high-density lipoproteins and a reduction in plasma cholesterol level. Plasma triglycerides levels are increased.[28]

2.4.2 Mechanism of action of estrogen:

Estrogen may act on the hypothalamic center to inhibit the secretion of gonadotropin releasing factor thereby preventing pituitary gonadotropin secretion and the resultant ovulation. The transport of ova through the tubules is either accelerated or inhibited so that the ova enter the unprepared uterus where it is degenerated or expelled. Administered estrogens also alter the delicate balance of estrogen and progesterone required for implantation of the blastocyte in the endometrium.

Estrogens also induce the formation of hormonal receptors necessary for the interaction of the different hormones. Most of the actions of estrogens are mediated by the activation of intracellular receptors.[29]

Estrogens exert their action by inducing specific physiologic response till the estrogenic receptors. The estrogenic receptors reside in the nucleus and the hydrophobic estrogenic compounds readily diffuse through the cellular and nuclear membrane to bind and subsequently activate the estrogenic receptors. The activated form of the estrogenic receptors can then stimulate the transcription of estrogen responsive genes.

2.4.3 The important biological activities of estrogen: [30]

a) Stimulation of growth of both the myometrium and endometrium.
b) Maintenance of a thick vaginal mucosa and indirectly the acidic vaginal pH.
c) Stimulation of cervical glands to secrete copious quantities of viscous mucous.
d) Stimulation of breast growth and development.

e) Deposition of subcutaneous fat which results in a characteristic feminine habitus.
f) Regulation of gonadotropin secretion including both "negative" and "positive" Feedback.
g) Sensitization of the ovaries to gonadotrapins and
h) Retardation of linear body growth in association with facilitation and epiphyseal closure.

2.5 Ovarian cycle: [26]

The normal reproductive years of the female are characterized by monthly rhythmic changes in the rates of secretion of the female hormones and corresponding changes in the ovaries and sexual organs as well. This rhythmic pattern is called the female sexual cycle (or less accurately, the menstrual cycle). The duration of the cycle averages 28 days. It may be as short as 20 days or as long as 45 days even in completely normal women, though abnormal cycle length is occasionally associated with decreased fertility.

The two significant results of the female sexual cycle are: First, only a single mature ovum is normally released from the ovaries each month. Second, the uterine endometrium is prepared for implantation of the fertilized ovum at the required time of the month.

After puberty, when FSH and LH from the anterior pituitary gland begin to be secreted in large quantity, the entire ovaries together with the follicle within them begin to grow.

Disturbance of the natural steroid hormone balance can successfully disorganize the co-ordinated events involved in ovulation, ovum transport and implantation. Thus, compounds possessing oestrogenic, progestation, anti-oestrogenic or anti progestational activity may also exhibit anti-fertility activity.[31]

2.5.1 Ovulation: [32, 33]

About 14th day of normal - 28 day menstrual cycle a mature graffian follicle ruptures to expel ovum. The egg and its associated cells enter the peritoneal activity briefly, and then pass in to fimbriated funnel of the oviduct. The fimbriae of the duct are close to the surface of the ovary at this time. The ovum must be fertilized soon after ovulation or it will degenerate, fragment and disappear. The union of the ovum and sperm takes place either before or immediately after the ovum enters the fimbriated extremity of the tube. The ovum reaches the uterus about 3 days after ovulation. Then embryo implanted in the endometrium about 6 days after ovulation. Prostaglandin's released by endometrial cells facilitate this process.

After ovulation, the ruptured follicle does not degenerate at once but is transformed temporarily into a glandular structure, the yellow body or corpus lutea.

2.5.2 Initiation of Ovulation: [26]

The initiating cause of ovulation is the large quantity of luteinizing hormone secreted by the anterior pituitary gland. The luteinizing hromone in turn causes rapid secretion of follicular steroid hormones containing a small amount of progesterone for the first time. Within a few hours two events occurs, both of which are necessary for ovulation.

1) The theca externa (the capsule of the follicle) begins to form proteolytic enzymes that cause dissolution of the capsular wall and consequent weakening of the wall, resulting in further swelling of the entire follicle and degeneration at the stigma.

2) Simultaneously, there is rapid growth of new blood vessels into the follicle wall, and at the same time prostaglandin's (local hormones that cuase vasodilation) are secreted in the follicular tissues. These two

effects in turn cause plasma tansudation into the follicle, which also contributes to follicle swelling. Finally, the combined follicle swelling and simultaneous degeneration of the stigma cause follicle rupture with evagination of the ovum.

2.6 The corpus luteum – The "Lueteal" phase of the ovarian cycle:

During the first few hours after expulsion of the ovum from the follicle, the remaining granulosa cells change rapidly into lutein cells. These grow to a diameter two or more times as large as the granulosa cells, and they become filled with lipid inclusions that give them a yellowish appearance. This process is called luteinization, and the total mass of cells together is called the corpus luteum. In the normal female, the corpus leuteum grow to approximately 1.5 cm.

The two types of ovarian sex hormones are the estrogens and the progestins, the most important of the estrogens is the hormone estradiol, and by far the most important progestin in progesterone. The estrogens mainly promote proliferation and growth of specific cells in the body and are responsible for development of most secondary sexual characteristics of the female. On the other hand, the progestins are concerned almost entirely with final preparation of the uterus for pregnancy and the breasts for lactation.[23]

2.7 Role of sperms in fertilization:

Semen is the complete discharge of the male during normal ejaculation. It consists of seminal plasma, spermatozoa and usually some cells cast off from the lining of the reproductive ducts and glands. Seminal plasma consists of the secretion of the prostate, seminal vesicles, bulbourethral glands and epididymis, the chief contribution being from the prostate and seminal vesicles. The seminal plasma serves as a food source and vehicle for the spermatozoa.

Testicular or epididymal sperm are inactive but quickly become active in seminal plasma (or saline). The role of the high content of hyaluronidase in sperm is not entirely understood but histochemical studies indicate that it breaks down the egg coating and aids the sperm in penetrating the egg.[34]

The production of spermatozoa occurs in the seminiferous tubules of the testis which are controlled by hormones from anterior pituitary gland. Leutinizing hormones (LH) stimulate the interstitial cells of leydig to produce testosterone. Testosterone is responsible for appearance of maintenance of secondary sex characteristics. The male accessory sex organs are the organs adopted for transfer of live spermatozoa from males to female. Sperm must remain in female tract for several hours to acquire ability to penetrate ovum-capacitation.[35]

As early as the 19th Century B.C. the Egyptians were mixing honey, natron (Sodium carbonate) and crocodile dung to form a vaginal contraceptive paste. During the middle ages, rock salt and alum were frequently used as vaginal contraceptives. The spermicidals must be inserted deep in to the vagina (usually with an applicator). They must be used just before intercourse and reused if intercourse is to be repeated. The ideal vaginal contraceptive must be non toxic and non irritating to both partners. The spermicidal agents also provide significant protection against venerial disease transmission. Modern spermicidal reagents or vaginal contraceptives fall in to 3 Categories.[36]

1. Surface active / sulfydryl binding agents

   Eg. (i) Non oxynol – I.U.S.P. (Delfen, Immolin)

   (ii) Otoxynol U.S.P. (Koromex)

2. Bactericides

   Eg. (i) Phenyl mercuric acetate (Lorophyn)

(ii) Benzenthonium chloride

(iii) Methyl enethonium chloride

(iv) Phenyl mercuric borate

3. Acids

Eg. Boric acid, Tartaric acid, Phenols etc

Contraception is literally the prevention of conception, but generally is taken to mean the prevention of pregnancy.[37]

2.8 Mechanism of steroid hormone action:

Estradiol, like other steroids, is thought to exert its action directly on the nucleus of the cell. As a consequence, an estrogen-response tissue must have estrogen receptor and nuclear acceptor sites to which activated receptor can bind. Upon entry into the cytosal of the cell (by diffusion), estradiol is bound to a specific receptor (ERc). In the cytoplasm, the estrogen receptor complex is activated (ERn) and translocated to the nucleus. This complex binds to acceptor sites in chromatin and enhances processes associated with differentiated functions of the responsive tissue, which include the production and utilization of messenger and other classes of RNA needed for the synthesis of constituent enzymic and secretory proteins, as well as the receptor itself. In some cells replication of DNA is also stimulated, followed by cell division. The concentration of estrogen receptors in most tissues is constitutive but in some instances it is increased by estradiol.

The concentration of progesterone receptors in the uterus and other progesterone response tissues is markedly increased by estrogen. In fact, one of the recognized actions of estradiol is to stimulate synthesis of progesterone receptors. The induction of progesterone receptors with estrogen can explain the synergistic action of these two hormones on the uterus.

2.9 Arachidonic acid pathway:

Arachidonic acid (AA) is an essential fatty acid consumed in the diet or derived from elongation and desaturation of dietary linoleic acid (LA).[38] AA metabolism is linked to signal transduction pathways that result in the activation of phospholipase C (PLC) and phospholipase A2 (PLA2).[39, 40, 41, 42] once AA is released from phospholipids it is oxidized by one of three different oxygenases.[38, 43] A certain cytochrome P-450 inserts a single oxygen atom into the double bond of AA to produce epoxy-arachidonic acids.[43] Lipoxygenases introduce one molecule of 0_2 into the carbon framework of AA to produce a series of isomeric hydroperoxy acids.[44] These hydroperoxy fatty acids are converted to hydroxy fatty acids, to leukotrienes (LTs) and to lipoxins.[38, 43, 44] Cyclooxygenase introduces two molecules of 0_2 into AA to form the hydroperoxy endoperoxide prostaglandin (PG) G2, which is reduced by the peroxidase activity to the hydroxy endoperoxide, PGH2.[38, 45, 46, 47] PGH2 is transformed to PGs, thromboxanes (TXs), prostacyclin, and malondialdehyde.[38] The physiological response to AA oxygenation in a given tissue is largely determined by the levels of PGH2-metabolizing enzymes in the cells making up that tissue. Each PG has its own range of biological activities. The LTs constitute a family of highly potent inflammatory mediators which are derived from the 5- lipoxygenase pathway of AA metabolism.[48, 49, 50] Eicosanoids are not stored but are synthesized on demand; therefore inhibitors of the various oxygenases and peroxidemetabolizing enzymes have instantaneous effects on eicosanoid levels.[51, 52]

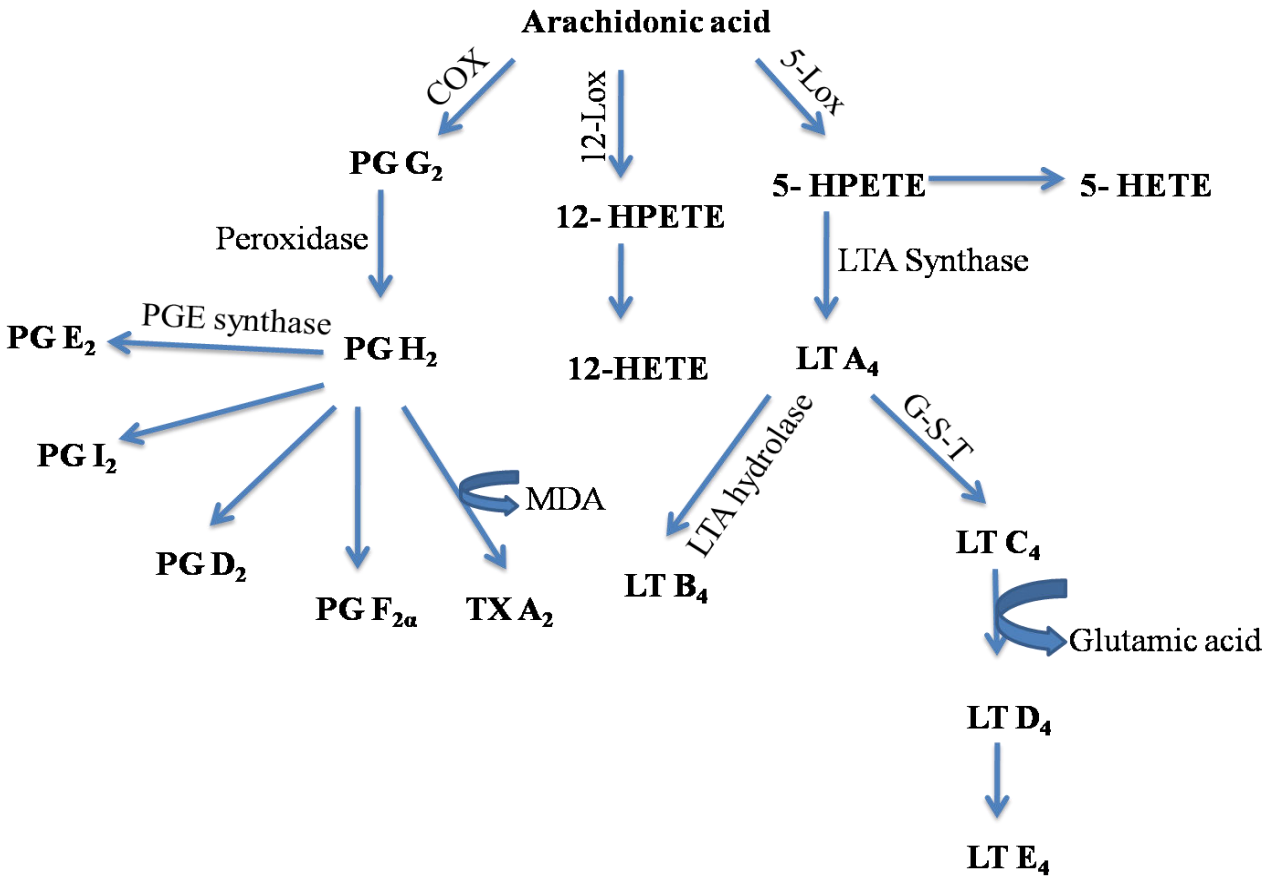


Figure 3: COX and LOX pathways of AA metabolism. Metabolites of COX pathway are called prostaglandins G_2 (PGG_2), E_2 (PGE_2), H_2 (PGH_2), D_2 (PGD_2), $F_{2\alpha}$ ($PGF_{2\alpha}$), thromboxane A_2 (TXA_2), malondialdehyde (MDA), and prostacyclin (PGI_2). Metabolites of the LOX pathway are called 12- and 5-hydroperoxyeicosatetraenoic acids (HPETEs), and their corresponding fatty acids (HETEs). Leukotriene A synthase (LTA synthase) generates the leukotrienes.

2.10 Biosynthesis of leukotrienes:

Unlike many other biologically active molecules, the eicosanoids are not stored preformed but are synthesized de novo from membrane phospholipids (arachidonic acid) through a cascade of enzymes. Arachidonic acid (5,8,11,14-cis-eicosatetraenoic acid) is found esterified in the sn-2 position, to cell

membrane phospholi- pids in a wide variety of mammalian cells.[53] The trigger for eicosanoid biosynthesis begins after trauma, infection and inflammation.[54] The initial step in the biosyn- thesis is a receptor-mediated influx of calcium ions that causes translocation of a phospholipase enzyme, cytosolic phospholipase A sub 2 (phospholipase A2), to the cell membrane.[55, 56, 57] The enzyme then catalyses the hydrolysis of the esterified form of arachidonic acid at its sn-2 position.[58] This selectively cleaves arachidonic acid from cell membranes.

There are three major pathways of metabolism from arachidonic acid as the substrate – the cyclo-oxygenase, lipoxygenase and epox- ygenase pathways.[59] The cyclo-oxygenase pathway leads to the formation of prostaglandins and thromboxanes while the lipoxygenase pathway is responsible for initiating the synthesis of leukotrienes. The third pathway (the epoxygenase pathway) is probably least important and also poorly understood; although it leads to the formation of prostaglandin epoxides which are thought to play a role in bleeding disorders. Only the second pathway (the lipoxygenase pathway) will be discussed in detail here.

The activity of phospholipase A2 is increased by a phospholipase A2-activating protein. This protein, when activated by cytokines e.g. tumour necrosis factor (TNF) and interleukin-1 (IL-1), can lead to arachidonic acid release and subsequent leukotriene formation. Arachidonic acid is then converted sequen- tially to 5-hydroperoxyeicosatetraenoic acid (5 HPETE) and then to LTA4 by a catalytic complex consisting of 5-LO and FLAP in the presence of adenosine triphosphate (ATP) and calcium ions (Ca2+).[60, 61] LTA4 is unstable and is either metabolized by LTA4 hydrolase to leukotriene B (LTB) sub 4 or conjugated to the tripeptide glutathione (γ-glutamylcysteinylglycine) by LTC4 synthase (a unique glutathione S-transferase) to form LTC4. In many biological systems, LTC4 is then rapidly converted to LTD4 via γ-glutamyl transpeptidase, which removes the amino acid glutamic acid from the glutathione moiety. LTD4 may then be converted

to LTE4 by the actions of a dipeptidase, which removes the glycine residue. This leaves cysteine as the only amino acid conjugated to the lipid portion of LTE4. Because LTC4, LTD4 and LTE4 contain cysteine, they have been designated cysteinyl leukotrienes. LTB4 can undergo further oxidation at the 20-carbon atom to the less active metabolites.

2.11 Distribution of leukotrienes:

The term leukotriene is derived from leukocytes because they were initially identified as products of leukocytes and the chemical structure contains three conjugated double bonds (triene). Apart from leukocytes, other myeloid derived cells can produce leukotrienes. The sites at which the leukotrienes are synthesized are determined by the cellular distribution of the enzymes controlling each stage of the biosynthetic pathway. The synthesis of LTA4 is limited to cells of the myeloid lineage, which are the primary sites of 5-LO. However, the enzymes determining the next step in the biosynthetic pathway (conversion to either LTB4 or the cysteinyl leukotrienes) are more widely distributed. This enables a much broader range of cells to act as leukotriene producers.

The distribution of 5-LO is limited to neutrophils, eosinophils, monocytes, macrophages, mast cells, basophils and B-lymphocytes.[62, 63] Considerable variation exists in both the type and the quantity of leukotrienes secreted in these cells. Apart from human monocytes and macrophages which produce both LTB4 and LTC4 all the other cells produce appreciable quantities of either LTB4 or LTC4 but not both. LTC4 is the principal 5-LO product released from activated eosinophils[64, 65, 66] as well as purified pulmonary mast cells while activated neutrophils secrete LTB4 as a product of 5- LO activity.[67]

The main sites of leukotriene production in the body by the myeloid derived cells that have been demonstrated are the lungs[68, 69, 70, 71] and the uterus.[72, 73, 74, 75, 76] The other potential sites of production are the kidneys[77] the skin of patients with urticaria, atopic dermatitis and psoriasis[78] the coronary circulation in patients with cardiac ischaemia and in the biliary system following human bile duct obstruction.[79] Increased concentrations of LTB4 and the cysteinyl leukotrienes are found in the sputum, bronchoalveolar lavage fluid, and urine of patients with cystic fibrosis. [80, 81, 82, 83] Some other conditions in which leukotriene production is increased are inflammatory bowel disease,[84] sickle cell disease and rheumatoid arthritis.[85] In the gut, LTB4 is found in colonic epithelial cells where synthesis is thought to take place and it is believed to promote infiltration by neutrophils of injured colonic mucosa in patients with inflammatory bowel disease. In patients with ulcerative colitis and Crohn's disease, the colonic mucosa contains significantly elevated concentrations of LTB4 compared with normal controls.[86, 87] LTB4 concentrations are also substantially elevated in the rectal dialysate fluid of this group of patients. [88, 89]

2.12 Leukotriene receptors:

Leukotrienes produce their biological effects by binding to and activating specific receptors. Two types of receptors for the cysteinyl leukotrienes (CysLT1 and CysLT2) have been demonstrated.[90] Recently, the molecular and pharmacological characteristics of cloned human CysLT1 receptor have been reported.[91] The CysLT1 receptor (designated HG55) is a glycosylated G-protein-coupled receptor (GPCR) [92] and it encodes a protein of 337 amino acids with a molecular mass of 38 549. All the three marketed cysteinyl leukotriene antagonists were demonstrated to be potent competitors with the CysLT1 receptor for binding of radiolabelled LTD4. Such characterization has so far not been elucidated for CysLT2 receptor. The receptor for the non-cysteinyl leukotriene (LTB4), is a seven-transmembrane- spanning protein known as the B leukotriene receptors (BLT).[93] These G-coupled receptors are members of

the rhodopsin-like receptor superfamily.[94]

The leukotriene receptors are located in the plasma membranes of smooth muscle cells and in other types of cells. [95, 96, 97] Most of the biological effects of the leukotrienes are mediated by the CysLT1 receptor.[98] These effects include the contrac- tion of human airway smooth muscle, chemotaxis, and increased vascular permeability.[99] LTC4 and LTD4 have equal capacity to stimulate smooth muscle contraction in the human lung in vitro by acting on CysLT1 receptors and the potency of LTE4 is lower by a factor of 10.[100] In the uterus, cysteinyl leukotrienes have also been demonstrated to stimulate myometrial smooth muscles (both circular and elongated myometrial smooth muscle) where specific binding sites have been demonstrated. Similar binding sites have also been demonstrated in the endometrial cells. The action of the CysLT2 receptor is less well defined although in humans, they have been shown to mediate contraction of pulmonary vascular smooth muscle.

Two distinct sites of high and low affinity for LTB4 exist on the surface of neutrophils. LTB4 is a very potent neutrophil chemotactic agent and may play a pivotal role in the induction of neutrophil–endothelial cell adherence as well as a mediator of inflammatory pain.

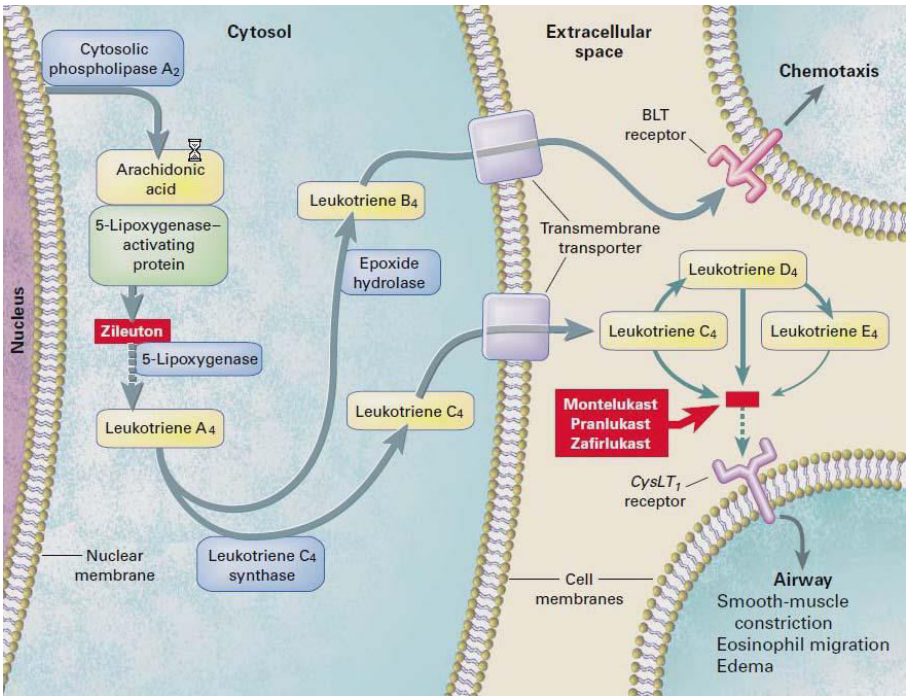


Figure 4: Biochemical Pathways of the Formation and Action of the Leukotrienes and Sites of Action of Leukotriene-Modifying Drugs.

2.13 Leukotrienes in the uterus:

The ability of uterine tissues to elaborate leukotrienes was first suggested in the guinea pig using the leukotriene receptor antago-nist FPL 55712, which inhibited antigen-induced uterine contrac- tions.[101] Although several other studies were conducted after this initial experiment to demonstrate increased amounts of leukotrienes in the endometrium and myometrial smooth muscles of patients with primary dysmenorrhoea little has been done on the potential therapeutic role of anti- leukotrienes in the management of this condition.

Leukotriene receptors were shown to be present in uterine tissues in the 1980s.[102] The number of these receptor sites for the cysteinyl leukotrienes has

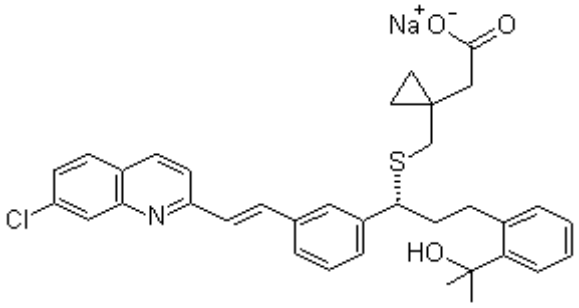
been demonstrated to be as high as that in lung. Incubation studies using light microscopic autoradiographs of non-pregnant human uterine and bovine lung tissues demonstrated the presence of specific LTC4 receptor sites in endometrial and myometrial smooth muscle cells. Experimental studies carried out by Demers et al. (1984) using human endometrium and myometrium demonstrated the capacity of these tissues to synthesize leukot- rienes. In the menstrual blood from women with primary dysmenor-rhoea compared with that in women without, significantly higher concentrations of LTC4 and LTD4 have been demonstrated. The presence of specific LTC4 binding sites in the myometrial cells have also been localized.[103]

2.14 DRUG PROFILE

MONTELUKAST SODIUM

Category : Antiasthmatic[104]

Structure



Montelukast sodium

Chemical Name: 2-[1-[[1-[3-[2-[(7-Chloro-2-quinolyl)] vinyl] phenyl]-3-[2-(1-hydroxy-1- methyl-ethyl)phenyl]-propyl]sulfanylmethyl]cyclopropyl]acetic acid sodium salt;[105]

Molecular formula: $C_{35}H_{35}ClNNaO_3S$

Molecular Weight: 608.17 g/mol

Physical appearance: white to off-white powder.

Solubility: Montelukast sodium is freely soluble in ethanol, methonol and water and practically insoluble in acetonitrile.[106]

Description: Montelukast sodium is a selective and orally active leukotriene receptor antagonists that inhibits the cysteinyl leukotriene CysLT1 receptor.[107]

Mechanism of action: The Cysteinyl leukotrienes (LTC4, LTD4, and LTE4) are products of arachidonic acid metabolism and are released from various cells, including mast cells and eosinophils.

These eicosanoids bind to cysteinyl leukotriene receptors (CysLT) found in the human airway. Cysteinyl leukotrienes and leukotriene receptor occupation have been correlated with the pathophysiology of asthma, including airway edema, smooth muscle contraction and altered cellular activity associated with the inflammatory process, which contribute to the signs and symptoms of asthma. It reduces the sputum eosinophil levels and causes bronchodilation.

Montelukast is as orally active compound that binds with high affinity and selectivity to the CysLT1 receptor (in preference to other pharmacologically important airway receptors such as the prostanoid, cholinergic or adrenergic receptor). Montelukast inhibits physiologic actions of LTD4 at the CystLT1 receptor without any agonist activity. It reduces the bronchial hyperesponsiveness, improves the lung function, and reduces bronchial inflammation.[108]

Available & recommended dosing:

Dose-finding studies indicate an optimum therapeutic dose of 10 mg/day in adults and adolescents.[109,110] Determination of the optimum daily dose of 5 mg in children aged 6–14 years[111] and 4 mg in infants as young as 6 months of age[112] is based on serum blood levels of the drug.. The 4 mg tablet formulation, unlike the others, should be administered either 1 h before or 2 h after food, as it may be better absorbed in an empty stomach.[113] The dosage for all formulations is one tablet/dose daily at bedtime.

Pharmacokinetics: Bioavailability (%) - 63

Protein Binding (%) - greater than 99

Elimination half life (hrs) - 2.7 to 5.5

Routes of administration - oral

Absorption: Montelukast sodium is well absorbed upon oral administration. Peak blood levels occur in about 2-2.5 hrs, with a half-life of 2.7-5.5 hrs.

Distribution: Montelukast sodium protein binding is greater than 99 %

Metabolism: Extensively metabolized. Plasma concentrations of metabolites are undetectable at steady state. CYP-450 3A4 and 2C9 are involved in metabolism.

Excretion: Montelukast sodium is eliminated by urine. Plasma clearance averages 45 ml/min. 86% recovered in feces and less than 0.2% in urine.

Pharmacodynamics: Montelukast causes inhibition of airway cysteinyl leukotriene receptors as demonstrated by the ability to inhibit bronchoconstriction due to inhaled LTD4 in asthmatics. Doses as low as 5 mg causes substantial blockage of LTD 4- induced bronchoconstriction. In clinical studies it decreased mean peripheral blood eosinophils approximately 13-15% from baseline compared with placebo over the double behind treatment periods.[114]

Drug Interactions: Phenobarbital, rifampicine.

Side Effects: These are in general few consisting headache and gastrointestinal disturbances. Few subjects developed Churg-Strauss syndrome (it's characterized by systemic vasculitis eosinophilia and a history of asthma, sinusitis and rhinitis). Cardiovascular - Cardiac complications, palpitations (post marketing).[115]

Usage: Montelukast sodium is used in prophylaxis and chronic treatment of asthma in patients 12 months of age and older; relief of symptoms of seasonal

allergic rhinitis in patients 2 yrs of age and older; relief of symptoms of perennial allergic rhinitis in patients 6 months of age and older.[116]

Diet: Reduces bioavailability.

Storage: Store at controlled room temperature (59° to 86°F). Protect from moisture and light.[117]

The estrous cycle in female rats is of 4–5 days duration and divided into four phases: proestrus (12–18 h) estrus (25–38 h), metestrus (5–8 h) and diestrus (47–58 h).[118]

2.15 Various stages of the estrus cycle in rats:

The estrus cycle is a cascade of hormonal and behavioural events, which are highly synchronised and repetitive. The short and precise estrus cycle of laboratory rat has been a useful model for reproductive studies. The laboratory rat is a spontaneous ovulating, non-seasonal, polyestrus animal. It ovulates every 4-5 days throught the year unless interrupted by pregnancy or pseudo-pregnancy.

Rats estrus cycles is roughly divided into four stages.

2.15.1 Proestrus: This is the beginning of a new cycle. The follicles of the ovary start to mature under the influence of gonadotropic hormones, and estrogen secretion start increasing. The vaginal smear is characterised by nucleated epithelial cells, the stage lasts for about 12 h.

2.15.2 Estrus: In this stage the uterus is enlarged and extended and extended due to fluid accumulation; estrogen secretion is at its peak. In the estrus stage, the smear shows presence of squamous cornified cells (hexagonal or pentagonal cells). The estrus stage is usually the period of heat and is characterised as a period of sexual receptivity, when the female allows

copulation. During this stage there is increased running activity. It lasts for 12 h.

2.15.3 Metestrus: The ovary contains corpora lutea which secrete profesterone. This stage is indicated by the presence of a mixture of cornified epithelial cells and leucocytes indicating the post-ovulatory stage and desquamation of the epithelial cells. The metestrus stage lasts for about 21 h.

2.15.4 Diestrus: the corpus lutea regress and the declining secretion of estrogen and progestrone cause regression of the uterus. The vaginal smear shows onlu leucocytes. This stage is the longest phase of the estrus cycle and has duration of about 57 h.

2.16 The experimental procedure for taking vaginal smears:

Holding the animal on the ventral side up, a drop of normal saline is inserted into the vagina with a pasteur pipette. Care must be taken to avoid damage or injury to the vagina so as to pseudo-pregnancy. The drop of normal saline should be aspirated and replaced several times. It is then transferred to a microscope slide and allow to dry. The smear are fixed by placing the slide in absolute alcohol for 5 s, allowing it to dry, and staining it with a 5% aqueous methylene blue solution for 10min. the excess stain is washed off with tap water, and the slide dried and observed using a low power microscope.[1]

2.17 Role of leukotriene in reproduction:

The lipoxygenase products (leukotrienes) have been demonstrated in many mammalian tissues including humans. They are widely distributed in the lungs, gut, uterus, kidneys, skin, heart and the liver. Their roles as mediators of inflammation have made them therapeutic targets. The potential use of lipoxygenase products in the management of primary dysmenorrhoea (especially in patients who are not responding to the traditional treatment using PG synthetase inhibitors) and possibly also in cases of endometriosis.[10]

Leukotrienes modulate steroidogenic cells functions, depending on the stage of the cycle. Leukotriene B (4) plays a luteotropic role stimulating P4 and PGE (2) secretions; LTC (4) stimulates the secretion of luteolytic factors and enhances the luteolytic cascade within BCL.[119]

2.18 Role of leukotriene in ovulation:

The concentrations of PGE, PGF, LTB, and LTC4/D/E increase in the ovary of PMSG-primed immature rats after the ovulatory process has been initiated by hCG administration. Since differences in the patterns of temporal changes of the various eicosanoids during ovulation are seen, we suggest that each eicosanoid plays a distinct role in the process of follicular rupture.[11]

Pharmacological inhibition of an alternative lipoxygenase (LOX) pathway has been shown to cause defective ovulation. The expression of ALOX5 and ALOX12 in the preovulatory follicle and that inhibiting the early phase LOX activity led to reduction in preovulatory PTGS2 induction and PGE2 production and thus defective ovulation. These findings may help to understand the mechanism for the involvement of the LOX pathway in LH-triggered ovulatory response, at least the induction of PTGS2 activity, in rats.[12]

LTB4 may play a role in the ovulatory pathways of the rat ovary and that LTB4 may carry out its effects via MMP-2.[13]

Rats treated with the LOX inhibitor NDGA (300 mg/kg bw) did not show ovulatory alterations. These data indicate that the characteristic alterations of follicle rupture induced by indomethacin, are also induced by selective COX-2 inhibitors, strengthening the contention that prostaglandins play a crucial role in the spatial targeting of follicle rupture at the apex.[120]

NDGA appears to be an inhibitor of ovulation in the rat. LTB 4 reversed the ovulation inhibition by NDGA, suggesting a role of this LT in intraovarian ovulatory changes. Steroidogenesis was marginally affected only when both the

lipoxygenase and cyclooxygenase pathways were inhibited and was associated with reduced PG levels. However, these findings must be couched in the understanding that neither the lipoxygenase nor the cyclooxygenase pathway is indispensable for ovulation to take place. This study further highlights the activities, interactions, overlap, and redundancy of arachidonic acid pathways supporting ovulation.[14]

2.19 Role of leukotriene in pregnancy:

Lipoxygenase metabolites may be involved in human parturition. Role for 5-LOX and FLAP in the control of parturition at term, and also suggest an involvement earlier in pregnancy.[19]

HSD11B2 in human placental trophoblasts is decreased by progesterone and increased by inhibition of endogenous LOX metabolites, and that a component of the effect of LOX metabolites on HSD11B2 is mediated by their stimulation of endogenous progesterone output.[20]

Lipoxygenase from human term placenta exhibits both dioxygenase and hydroperoxidase activities, and this enzyme represents an important pathway for chemical oxidation in the placentas of non-smoking women.[121]

LT action in the bovine reproductive tract is dependent on LT type: LTB(4) is luteotropic during the estrous cycle and supports early pregnancy, whereas LTC(4) is luteolytic, regarded as undesirable in early pregnancy. LTs are produced/secreted in the CL tissue, influence prostaglandin function, and serve as important factors during the estrous cycle and early pregnancy in cattle.[122]

Paracrine loop between LTB4 and oxytocin is lacking in fetal membranes and amnion at term pregnancy. Oxytocin exerts a stimulatory effect on PGE2 release by both fetal membranes and amnion. The interrelationships

between oxytocin and the different eicosanoids in the above tissues seem to be highly selective.[123]

The affinity of PGF2 alpha to the uterus and the role of endogenous PGs in the maintenance of pregnancy by influencing connective tissue metabolism.[124]

The arachidonate lipoxygenase pathway is highly active before labor; 2) LTB4 might play a role in the regulation of PGE2 production.[125]

Activation of protein kinase C can result in enhanced production of arachidonate lipoxygenase metabolites that may have actions on the parturient process.[126]

LTB4 may play an important role in both placental separation and uterine involution in cattle and LTB4 synthesis may be modulated by endocrine and bacterial factors.[127]

Nordihydroguaiaretic acid, a leukotriene inhibitor, reduced PGE release as well as 6-keto-PGF1 alpha.[128]

A critical role for LTB4 in averting NK-mediated early spontaneous fetal resorption.[129]

A large variation of 12-HETE production it remains to clarify its role in the cervix.[130]

An activation of the arachidonate lipoxygenase pathway in these tissues during labor. Oxytocin could play a regulatory role in this process.[131]

The potentiation of contraction force to oxytocin by CRH is dependent on prostaglandins, probably PGF2 alpha and that leukotrienes, generated via the lipoxygenase pathway are not involved.[132]

5-HETE and LTC4, but not PGF2 alpha, are associated with uterine contractility after preterm intrauterine surgery. Surprisingly, AF PGF2 alpha levels were nondetectable for 1 to 2 weeks after surgery. 5-HETE and LTC4 are present in higher concentrations than PGF2 alpha in AF. 5-HETE, LTC4 and PGF2 alpha all increase with the onset of labor. AF concentrations of 5-HETE and LTC4 are significantly higher than PGF2 alpha before and during term and preterm labor. Labor can occur with suppressed PGF2 alpha levels, but with increasing 5-HETE and LTC4 levels. These data suggest that 5-HETE and LTC4 are important components of the parturitional process, and they challenge the dogma that PGs are the universal mediators of labor.[133]

5-HETE and LTC4, but not PGF2 alpha, are associated with uterine contractility after preterm intrauterine surgery. Surprisingly, amniotic fluid PGF2 alpha, levels were nondetectable for 1 to 2 weeks after surgery. 5-HETE and LTC4 are present in higher concentrations than PGF2 alpha in amniotic fluid. 5-HETE, LTC4, and PGF2 alpha all increase with the onset of labor. Amniotic fluid concentrations of 5-HETE and LTC4 are significantly higher than those of PGF2 alpha before and during term and preterm labor. Labor can occur with suppressed PGF2 alpha levels but with increasing 5-HETE and LTC4 levels. These data suggest that 5-HETE and LTC4 are important components of the parturitional process.[134]

Both PGs and LTs are required for the induction and progression of decidualization.[21]

There is an interaction between LTs and PGs in the induction of the uterine decidual response.[135]

Metabolism of arachidonic acid (AA) via cyclooxygenase to prostaglandins and thromboxanes and via lipoxygenase to hydroxyeicosatetraenoic acids (HETEs) and leukotrienes is an integral part of both the acute and chronic inflammatory reaction in the lung or uterus.[136]

2.20 Role of leukotriene in fertilization

Mouse and human spermatozoa require cysteinyl leukotriene activity for both fertilization and oocyte penetration.[18]

2.21 Role of leukotriene in implantation

LTs in uterine functions during implantation processes and decidualization has been reported.[15, 16] A selective inhibitor of 5-lipoxygenase enzyme may not impair the implantation in mice indicating a doubt about the involvement of 5-lipoxygenase products in implantation.[137]

The presence of both the cyclooxygenase and the lipoxygenase pathways in the preimplantation rabbit uterus and blastocyst, their differential operation in various compartments of the uterus on various days of early pregnancy suggests an integrated role for these mediators in embryo-uterine interaction during implantation.[17]

CHAPTER-3

OBJECTIVES

The objective of the study was to evaluate the "Anti-fertility activity of montelukast, a cysteinyl leukotriene receptor antagonist in female albino *Wistar* rats" with the following headings.

- To evaluate the effect of montelukast on pre-implantation in albino *Wistar* rats
- To evaluate the effect of montelukast on post-implantation in albino *Wistar* rats
- To evaluate the effect of montelukast on anti-fertility activity in albino *Wistar* rats

CHAPTER-4

MATERIALS AND METHODS

4.1 Animals

Inbred female (100-150 g) and male (150-250 g) albino Wistar rats were obtained from Swamy Vivekanandha College of pharmacy, animal house and maintained under standard conditions at 21±1^{o} C and 50-60% relative humidity with a photoperiod of 12 h light: 12 h dark. The animals were fed with standard pellet diet and water *ad libitum*. The protocols received prior approval from the Institutional Animal Ethical Committee (SVCP/IAEC/M.Pharm/06/2011) and experiments were conducted in accordance with guidelines set by the CPCSEA (Committee for the purpose of control and supervision of experiments on animals), India.

4.2 Drugs:

A gift sample of Montelukast obtained from (Ranbaxy Labs Ltd., Mumbai) was used for the study.

4.3 Chemicals:

Carboxy methyl cellulose (Loba Chemie Pvt. Ltd., Mumbai).

Betadine (G.S.Pharmbutor Pvt. Ltd., Rudrapur).

Neosporin (Glaxo Smithkline Pharmaceutical Ltd. Bangalore).

4.4 Instruments:

Compound microscope (I.R.Technology Services Pvt. Ltd., Mumbai).

Surgical catguts (*Johnson & Johnson* Pvt.Ltd., Mumbai).

Pipettes (*Riviera* Glass Pvt. Ltd., Mumbai).

Silk thread (Neelam Thread Pvt. Ltd., Delhi).

4.5 Anti-Implantation Activity

Vaginal smears of the female were taken daily for assessment of their oestrus cycle. Adult female albino Wistar rats of established fertility in pro-oestrus and oestrus were housed with mature male rats of established fertility (three females for one male). On mating days, females of the appropriate weight were placed in cages with males for approximately 14 h. Each female was examined for the presence of spermatozoa in the early morning vaginal smears. The first presence of sperms in the vaginal smear was taken as day 1 of pregnancy. In rats, implantation occurs on day 5 of pregnancy.

The female rats which had mated were then separated and caged singly. They were divided into four different groups consisting of six animals each.

The various groups were treated as follows:

The drug were administered orally once daily through gastric gavage from 1-7 days of pregnancy and 7-21 days of pregnancy

Group I – received the 1% w/v CMC- water suspension and served as control (p.o. daily).

Group II - received Montelukast 1mg/kg body weight in 1%w/v CMC in water (p.o. daily).

Group III- received Montelukast 10mg/kg body weight 1%w/v CMC in water (p.o. daily).

Group IV- received Montelukast 20mg/kg body weight 1%w/v CMC in water (p.o. daily).

On day 10 of pregnancy, the animals underwent a laparotomy under light ether anesthesia in semisterile condition. The implants present in both uterine horns as well as the corpora lutea (CL) on each ovary were counted. The animals were allowed to complete the gestation period (21-23 days) and the number of litters delivered were recorded. The percentages of pre- and post-implantation loss and the anti- fertility activity were calculated using the following formula.[138]

$$\% \text{ Pre- implantation loss} = \frac{\text{number of CL} - \text{number of implants}}{\text{Number of CL}} \times 100$$

$$\% \text{ Post- implantation loss} = \frac{\text{number of implants} - \text{number of litters}}{\text{number of implants}} \times 100$$

$$\% \text{ Anti-fertility activity} = \frac{\text{number of CL} - \text{number of litters}}{\text{number of CL}} \times 100$$

4.6 Procedure for laparotomy

The animal was lightly anesthetized with ether and limbs were tied to a rat board (waxed) with the ventral side up. The hairs on the area around the midline abdominal region were clipped with curved scissor and the region was cleaned with 70 % alcohol. An incision of 2 cm length was made along the midline to expose the viscera. The superficially lying coils of ileum were lifted to expose the two uterine horns. The horns were examined for implantation sites. Implants were visible as clear swellings on the uterine horns giving the

uterine tube a beaded appearance. Embryos with bright red dish aspect and a clear margin were considered to be healthy. Those with dull blue color with no clear margin and orientation with some exudates were considered resorbing.[139] The number of implants and resorption sites per horn were counted. The ovaries, which lie on the upper end of the uterine horns, show corpora lutea as reddish yellow spots over the surface. The number of corpora lutea present on each ovary was also noted.

After counting, the organs were replaced back. A small quantity of Neosporin powder was sprinkled over the organs to prevent any infection. The incision through muscular layer was closed with continuous suture using absorbable catguts and skin layer with continuous sutures using silk thread. An antiseptic, povidone iodine solution was applied on the sutured area after wiping with 70 % alcohol. The animal was maintained on light ether anesthesia throughout the experiment. After laparotomy the rats was transferred to a warm place till it recovered from the anesthesia.

4.7 Statistical analysis

The values are expressed in mean ± SEM. One way ANOVA followed by Tukey's multiple comparison Test was used to analyse the effect of different dose of drug when compared to control, with the help of Graph Pad Instat software, version 3.01. $P<0.05$ considered as significant.

CHAPTER-5

RESULTS

5.1 The Effect of Montelukast on Implantation:-

5.1.1Anti-implantation activity of montelukast when treated with 1-7 days of pregnancy

The LOX inhibitor montelukast elicited significant pre-implantation loss at dose levels of 20 mg/kg ($p<0.01$) and the anti-fertility activity, at dose levels of 10 mg/kg ($p<0.05$), 20 mg/kg ($p<0.01$), when compared to control group (Table: 1-4, Figure: 1).

Table 1: Pre-implantation, post-implantation and anti-fertility activity of control group (1% w/v CMC 10 ml/kg)

S.NO.	BODY WEIGHT	NO. OF CORPORA LUTEA	NO. OF IMPLANTS	NO. OF LITTERS	% PRE-IMPLANTATION LOSS	% POST-IMPLANTATION LOSS	% ANTI-FERTILITY ACTIVIY
1	180	10	7	6	30	14.28	40
2	170	12	9	8	25	11.11	33.33
3	190	13	8	4	38.46	50	69.23
4	200	14	9	7	35.71	22.22	50
5	150	12	7	5	41.66	28.57	58.33
6	170	11	10	9	9.09	10	18.1818
MEAN	176.666	12	8.33	6.5	29.9866	22.696	44.845
± SEM	7.149	0.5774	0.494	0.7638	4.839	6.182	7.461
± SD	17.512	1.414	1.211	1.871	11.852	15.143	18.276

Table 2: Pre-implantation, post-implantation and anti-fertility activity of montelukast (1 mg/kg)

S.NO.	BODY WEIGHT	NO. OF CORPORA LUTEA	NO. OF IMPLANTS	NO. OF LITTERS	% PRE-IMPLANTATION LOSS	% POST-IMPLANTATION LOSS	% ANTI-FERTILITY ACTIVITY
1	180	15	0	0	100	0	100
2	150	15	12	11	20	8.33	26.66
3	200	15	0	0	100	0	100
4	180	13	11	2	15.38	81.81	84.61
5	160	11	8	7	27.27	12.5	36.36
6	200	9	1	0	88.88	100	100
MEAN	178.33	13	5.3333	3.333	58.58833	33.773	74.605
± SEM	8.333	1.033	2.305	1.892	17.014	18.325	13.900
± SD	20.41	2.530	5.645	4.633	41.676	44.888	34.048

Table 3: Pre-implantation, post-implantation and anti-fertility activity of montelukast (10 mg/kg)

S.NO.	BODY WEIGHT	NO. OF CORPORA LUTEA	NO. OF IMPLANTS	NO. OF LITTERS	% PRE-IMPLANTATION LOSS	% POST-IMPLANTATION LOSS	% ANTI-FERTILITY ACTIVITY
1	150	11	0	0	100	0	100
2	200	11	10	3	9.09	70	72.72
3	170	14	11	7	21.42	36.36	50
4	160	13	12	2	7.69	83.33	84.61
5	170	10	2	0	80	100	100
6	180	12	5	1	58.33	80	91.66
MEAN	171.666	11.833	6.666	2.1666	46.088333	61.615	83.165
± SEM	5.943	0.6009	2.060	1.078	15.977	15.042	7.853
± SD	15.723	1.472	5.046	2.639	39.136	36.844	19.236

Table 4: Pre-implantation, post-implantation and anti-fertility activity of montelukast (20 mg/kg)

S.NO.	BODY WEIGHT	NO. OF CORPORA LUTEA	NO. OF IMPLANTS	NO. OF LITTERS	% PRE-IMPLANTATION LOSS	% POST-IMPLANTATION LOSS	% ANTI-FERTILITY ACTIVITY
1	200	9	0	0	100	0	100
2	180	13	0	0	100	0	100
3	190	9	0	0	100	0	100
4	150	14	0	0	100	0	100
5	170	13	0	0	100	0	100
6	170	11	0	0	100	0	100
MEAN	176.666	11.5	0	0	100	0	100
± SEM	7.149	0.8851	0	0	0	0	0
± SD	17.51	2.168	0	0	0	0	0

Table 5: Shows anti-implantation activity of montelukast treated for 1-7 days of pregnancy at different dose levels of 1, 10 and 20 mg/kg body weight

S.NO.	TREATMENT	% PRE-IMPLANTATION LOSS (mean ± SEM)	% POST-IMPLANTATION LOSS (mean ± SEM)	% ANTI-FERTILITY ACTIVIT(mean ± SEM)
1	Control	29.986 ± 4.839	22.696 ± 6.182	44.845 ± 7.461
2	Montelukast (1 mg/kg)	58.588 ± 17.014	33.773 ± 18.325	74.605 ± 13.900
3	Montelukast (10 mg/kg)	46.088 ± 15.977	61.615 ± 15.042	83.165 ± 7.853*
4	Montelukast (20 mg/kg)	100 ± 0.000**	00 ± 0.000	100 ± 0.000**

n=6; The values are expressed as mean ± SEM; *P< 0.05, **P<0.01 when compared to control groups (One way ANNOVA followed by Tukey's multiple comparison Test).

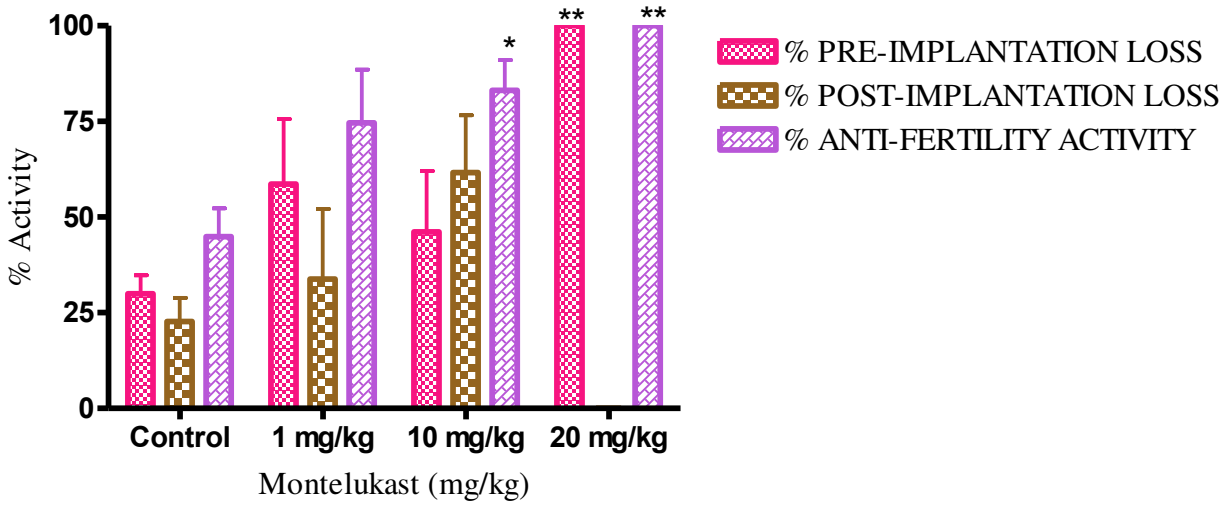


Figure 1: Pre- and post-implantation loss (%) and anti-fertility activity (%) of montelukast when administered orally at various dose levels to female albino *Wistar* rats from days 1 to 7 of pregnancy. Values are expressed as mean ± SEM; *P<0.05, **P<0.01 when compared to control group (n=6).

5.1.2 Anti-implantation activity of montelukast when treated 7-21 days of pregnancy

The LOX inhibitor montelukast elicited significant pre-implantation loss at dose levels of 1 mg/kg (p<0.05), 10 mg/kg (p<0.001), 20 mg/kg (p<0.001) and the anti-fertility activity, at dose levels of 1 mg/kg (p<0.001), 10 mg/kg (p<0.001) and 20 mg/kg (p<0.001), when compared to control group (Table: 6-9, Figure: 2).

Table 6: Pre-implantation, post-implantation and anti-fertility activity of control group (1% w/v CMC 10 ml/kg)

S.NO.	BODY WEIGHT	NO. OF CORPORA LUTEA	NO. OF IMPLANTS	NO. OF LITTERS	% PRE-IMPLANTATION LOSS	% POST-IMPLANTATION LOSS	% ANTI-FERTILITY ACTIVITY
1	180	10	8	6	20	25	40
2	160	14	11	7	21.428	36.36	50
3	170	9	8	4	11.11	50	55.55
4	170	12	10	6	16.66	40	50
5	190	10	8	5	20	37.5	50
6	160	15	12	8	20	33.33	46.66
MEA	171.6	11.666	9.5	6	18.199	37.031	48.701

N	66						
± SEM	4.773	0.988	0.7188	0.5774	1.558	3.347	2.096
± SD	11.690	2.422	1.761	1.414	3.815	8.199	5.135

Table 7: Pre-implantation, post-implantation and anti-fertility activity of montelukast (1 mg/kg)

S.NO.	**BODY WEIGHT**	**NO. OF CORPORA LUTEA**	**NO. OF IMPLANTS**	**NO. OF LITTERS**	**% PRE-IMPLANTATION LOSS**	**% POST-IMPLANTATION LOSS**	**% ANTI-FERTILITY ACTIVITY**
1	150	11	6	0	45.45	100	100
2	170	13	8	1	38.46	87.5	92.3076
3	190	12	9	2	25	77.77	83.33
4	160	13	0	0	100	0	100
5	160	12	0	0	100	0	100
6	200	11	8	2	27.27	75	81.8181
MEAN	171.666	12	5.16666	0.8333	56.03	56.711667	92.909283
± SEM	7.923	0.3651	1.682	0.4014	14.233	18.287	3.493
± SD	19.408	0.8944	4.119	0.9832	34.864	44.793	8.555

Table 8: Pre-implantation, post-implantation and anti-fertility activity of montelukast (10 mg/kg)

S.NO.	BODY WEIGHT	NO. OF CORPORA LUTEA	NO. OF IMPLANTS	NO. OF LITTERS	% PRE-IMPLANTATION LOSS	% POST-IMPLANTATION LOSS	% ANTI-FERTILITY ACTIVITY
1	150	13	3	1	76.9230	66.6666	92.3076
2	160	14	0	0	100	0	100
3	190	12	0	0	100	0	100
4	200	15	0	0	100	0	100
5	200	9	0	0	100	0	100
6	150	14	0	0	100	0	100
MEAN	175	12.8333	0.5	0.16666	96.15833	11.1111	98.717933
± SEM	9.916	0.8724	0.5000	0.1667	3.846	11.111	1.282
± SD	24.290	2.137	1.225	0.4082	9.421	27.217	3.140

Table 9: Pre-implantation, post-implantation and anti-fertility activity of montelukast (20 mg/kg)

S.NO.	BODY WEIGHT	NO. OF CORPORA LUTEA	NO. OF IMPLANTS	NO. OF LITTERS	% PRE-IMPLANTATION LOSS	% POST-IMPLANTATION LOSS	% ANTI-FERTILITY ACTIVITY
1	160	13	0	0	100	0	100
2	170	11	2	0	81.81	100	100
3	200	11	2	0	81.81	100	100
4	150	12	0	0	100	0	100
5	180	11	0	0	100	0	100
6	200	13	0	0	100	0	100
MEAN	176.666	11.8333	0.666	0	93.93667	33.333	100
± SEM	8.433	0.4014	0.4216	0	3.835	21.082	0
± SD	20.656	0.9832	1.033	0	9.393	51.640	0

Table 10: Shows anti-implantation activity of montelukast treated for 7-21 days of pregnancy at different dose levels of 1, 10 and 20 mg/kg body weight

S.NO.	TREATMENT	PRE-IMPLANTATION LOSS (mean ± SEM)	POST-IMPLANTATION LOSS (mean ± SEM)	ANTI-FERTILITY ACTIVITY (mean ± SEM)
1	Control	18.199 ± 1.558	37.031 ± 3.347	48.701 ± 2.096
2	Montelukast (1 mg/kg)	56.03 ± 14.233*	56.711667 ± 18.287	92.909283 ± 3.493***
3	Montelukast (10 mg/kg)	96.15833 ± 3.846***	11.1111 ± 11.111	98.717933 ± 1.282***
4	Montelukast (20 mg/kg)	93.93667± 3.835***	33.333 ± 21.082	100 ± 000***

n=6; The values are expressed as mean ± SEM; *P< 0.05, ***P<0.001 when compared to control groups (One way ANNOVA followed by Tukey's multiple comparison Test).

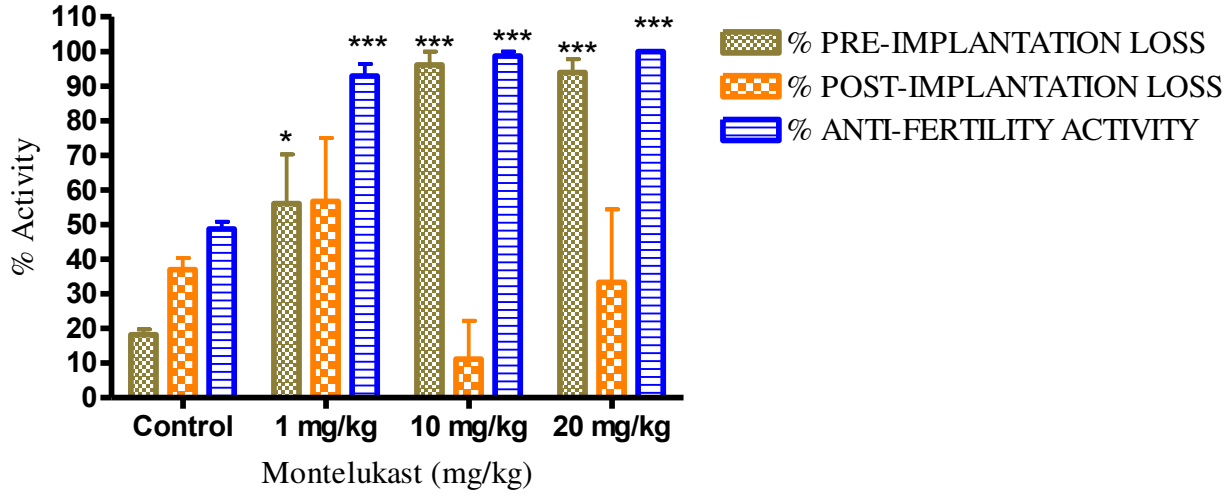


Figure 2: Pre- and post-implantation loss (%) and anti-fertility activity (%) of montelukast when administered orally at various dose levels to female albino *Wistar* rats from days 7 to 21 of pregnancy. Values are expressed as mean ± SEM; *P<0.05, ***P<0.001 when compared to control group (n=6).

CHAPTER-6

DISCUSSION

India possess 16 percent of the world's population on 2.4 percent of the globe's land area. Thus the most important problem India facing today is the size and growth of its population. The census 2001 has shown that the population of India was 102.70 crores as on 1st March 2001. In the 50 years since 1951 the population of the country has increased from 36.11 crores to 102.70 crores. India's population is growing very fast the pressure of numbers on the natural resources of the country is bound to grow. The density of population has increased from 117 persons per sq.km in 1951 to 324 persons in 2001, which may worsen the already very poor social and demographic indicators in this country. [140]

Arachidonic acid is metabolized mainly by lipoxygenases (LOX, which includes 5-LOX, 12-LOX, and 15-LOX) and cyclooxygenase (COX, which includes COX-1, COX2). 5-lipoxygenase is the first committed enzyme in the LOX pathway leading to the production of 5-hydroperoxyeicosatetraenoic acid (5-HPETE) with the subsequent production of 5-HETE and leukotrienes, which includes LTA4, LTB4, LTC4, LTD4, and LTE4.[141] Targeting upstream enzymes affects multiple downstream pathways, and may produce adverse effects by disrupting the balance between different AA-metabolizing pathways. It may be more advisable to target downstream enzymes or receptors.[142]

The lipoxygenase products (leukotrienes) have been demonstrated in many mammalian tissues including humans.[10] Leukotrienes are involved in various stages of reproduction including ovulation,[11, 12, 13, 14] implantation,[17, 143,144] decidulation,[21] parturition.[19]

Pakrasi *et al.,* (1985) reported that the presence of the lipoxygenase pathways in the pre-implantation rabbit uterus and blastocyst, their differential

operation in various compartments of the uterus on various days of early pregnancy suggests an integrated role for these mediators in embryo-uterine interaction during implantation.[17] Leukotrienes could be important for uterine preparation for implantation and/or implantation per se.[143] Lipoxygenase product might have a role in implantation.[144]

Mouse and human spermatozoa require cysteinyl leukotriene activity for both fertilization and oocyte penetration.[18] Lipoxygenase metabolites may be involved in human parturition. Role for 5-LOX and FLAP in the control of parturition at term, and also suggest an involvement earlier in pregnancy.[19] LTs are required for the induction and progression of decidualization.[21]

If these role of leukotriene in normal implantation are blocked it may negatively affect the implantation. Based on these hypothesis we have selected montelukast, a cysteinyl leukotriene receptor antagonists for evaluating anti-fertility activity in albino *Wistar* rats.

Montelukast sodium is used primarily for the treatment of asthma, Chronic Obstructive Pulmonary Disease (COPD) and in relieving the symptoms of allergic rhinitis. It is an orally active compound that binds with high affinity and selectivity to the CysLT1 receptor. Montelukast inhibits physiological actions of LTD_4 at the CystLT1 receptor.[145]

In the present study the oral administration of leukotrienes receptor antagonist montelukast was tested for anti-fertility properties in three doses of 1mg/kg (conversion from adult human dose to animal dose), 10mg/kg and 20 mg/kg body weight of rats[146, 147] for 1 to 7 days and 7 to 21 days of pregnancy.

Days 1-7 of treatment were selected because they cover the entire implantation period. Days 1-3 are the days of the preparation of the endometrium for the implantation of the blastocyst, when the uterine blood flow increases rapidly due to increased permeation of endometrial capillaries.[148] Afternoon of day 4 is believed to be the time of the "estrogen

surge", which might be responsible for implantation on day 5. Days 6-7 are very early stages of post- implantation period.[149] Days 7-21 is post-implantation period.

In 1 to 7 days the rats treated with montelukast 10 mg/kg showed significant ($p<0.05$) % anti-fertility activity when compared to control group. The animal's treated with montelukast 20 mg/kg showed significant ($p<0.01$) % pre-implantation loss and % anti-fertility activity when compared to control group (Table: 1-5, Figure: 1). In this study treatment of 1-7 days, montelukast showed significant anti-fertility activity in a dose dependent manner.

In 7 to 21 days the rats treated with montelukast 1 mg/kg showed significant ($p<0.05$) % pre-implantation loss when compared to control group. The animals treated with montelukast 10 mg/kg, 20 mg/kg showed significant ($p<0.001$) % pre-implantation loss when compared to control group. The experimental group treated with montelukast 1 mg/kg, 10 mg/kg, 20 mg/kg showed significant ($p<0.001$) % anti-fertility activity when compared to control group (Table: 6-10, Figure: 2). In this study treatment of 7-21 days montelukast showed significant anti-fertility activity in a dose dependent manner.

Pakrasi, (1997) have reported that a selective inhibitor of 5-lipoxygenase enzyme may not impair the implantation in mice indicating a doubt about the involvement of 5-lipoxygenase products in implantation.[11] But in our study results showed dose dependent anti-implantation activity of montelukast a cystenyl leukotrienes receptor antagonist in female albino *Wistar* rats.

If an ideal contraceptive is available, that would be 100% effective, safe and easy to use and its effect would be reversible.[1] We showed that the LOX-inhibitor montelukast 20 mg/kg achieved 100% anti-fertility activity in both 1-7 days and 7-21 days study treatment.

Our study revealed that montelukast satisfying the one of the criteria of ideal contraceptive. Our results also confirm that leukotriene are involved in the process of implantation. We interpret our results as confirmation that leukotrienes produced in the endometrium are involved in implantation in the rat and that the drug we administered act at the endometrial level. To confirm this interpretation it would be essential to document that the treatments did not cause endocrine changes that interfere with implantation of pregnancy. For instance, an increase in oestradiol would accelerate egg transport through the oviduct and reduce the number of implanted embryos. Inhibition of prolactin secretion and subsequent luteolysis with decreased progesterone production would also cause pre and post implantation loss. An additional approach could consist of demonstrating that exogenous progesterone does not reverse the action of drugs.

We consider that strength of our anti-fertility study includes that results were obtained by direct observation. Anti-implantation research is a promising field of WHO contraceptive research and development programmes. Since we studied the anti-implantation activity of montelukast, it may add good scientific knowledge to the contraceptive research.

Our study suggested that montelukast can be used as an anti-fertility agent, since it produced 100% anti-fertility activity. However further studies needed to establish the safety and efficacy of montelukast in pregnant animals. If the efficacy and safety is established in animals, we can further proceed to establish its efficacy and safety in humans by suitable clinical trials.

Our study also suggested that the cysteinyl leukotriene receptor antagonist montelukast can be avoided in pregnant women and the women with reproductive age groups. It is also suggested that proper pharmacovigilance studies can be carried out in montelukast to establish its safety in pregnant women and proper warning can be done on the label.

CHAPTER-7

CONCLUSION

Montelukast, a cysteinyl leukotrienes receptor antagonist posses 100% anti-implantation activity in albino *Wistar* rats. Our results indirectly confirms the involment of leukotrienes in implantation. Our results suggested that montelukast can be used as an anti-fertility agent after confirming its safety and efficacy in humans.

CHAPTER-8

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